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Superovulation and embryo transfer in Holstein cattle using sexed sperm

H. Hayakawa [a,*], T. Hirai [b], A. Takimoto [a], A. Ideta [c], Y. Aoyagi [c]

[a] *Genetics Hokkaido, Shimizu 089-0103, Japan*
[b] *Genetics Hokkaido, Nakashibetsu 086-1150, Japan*
[c] *Zen-Noh Embryo Transfer Center, Kamishihoro 080-1407, Japan*

Abstract

The use of sexed bull sperm in multiple ovulation and embryo transfer (MOET) programs for Holsteins was evaluated for (1) heifers housed at a commercial embryo transfer (ET) facility (Experiments 1 and 2), and (2) heifers and cows on dairy farms (Experiment 3). In Experiment 1, superstimulated heifers were inseminated with 5×10^6 sexed (X-sorted; $n = 5$) or unsexed ($n = 5$) frozen-thawed sperm from one bull at 12 and 24 h after estrus detection. No difference was observed in the rates of transferable embryos (53.4% vs 68.1%), degenerate embryos (24.8% vs 26.6%) and unfertilized ova (21.8% vs 5.3%) between sexed and unsexed sperm, respectively, except for the percent of female transferable embryos diagnosed by embryo sexing (100% vs 49.3%, $P < 0.0001$). In Experiment 2, donors were inseminated twice with 5×10^6 sexed unfrozen sperm ($n = 10$) or sexed frozen-thawed sperm ($n = 9$). Embryo production rates for both treatments were similar to that observed on a commercial ET facility using unsexed sperm. Pregnancy rates for frozen-thawed embryos were similar for sexed and unsexed sperm (70.4% vs 72.4%, respectively). In Experiment 3, 99 flushes were conducted using sexed frozen-thawed sperm from nine bulls but an overall statistical analysis was not completed because the use of bulls was not balanced. However, for one bull with balanced usage, the rate of transferable embryos was higher in heifers than in cows ($P < 0.05$) inseminated twice with 5×10^6 sperm/dose (10×10^6 total). We concluded that the use of sexed frozen-thawed sperm (≥90% X-sperm biased and 10×10^6 total sperm) may be economically viable for commercial MOET programs in Holstein heifers.

Keywords: Holstein cattle; Sexed sperm; Superovulation; Artificial insemination; Embryo transfer

1. Introduction

In the past decade, sexed bovine sperm has emerged as a complement to artificial insemination (AI) and has been accepted widely by markets [1]. Since the supply of sexed sperm is limited and costly, there is great interest to use it in multiple ovulation and embryo transfer (MOET) programs as opposed to inseminating single ovulating cattle [2–6]. While low dose sexed sperm (2×10^6 per dose) has been commercially available [1], Panarace et al. [2] reported successful embryo production when superstimulated heifers and cows were inseminated using 10×10^6 sexed frozen-thawed sperm per dose at 0, 12 and 24 h after estrus detection. Sartori et al. [3] reported no difference in transferable embryo rates between superovulated

* Corresponding author at: Sexing Laboratory, Genetics Hokkaido Assoc., Aza Simizu 5-18, Shimizu, Hokkaido 089-0103, Japan. Tel.: +81 156 62 2158; fax: +81 156 62 2150.
E-mail address: hayakawa@gh-assoc.ne.jp (H. Hayakawa).

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Holstein heifers inseminated with 20×10^6 sperm once (12 h after estrus detection) or 10×10^6 sperm twice (12 and 24 h after estrus detection) using sexed frozen-thawed sperm, but the rates were lower than that of unsexed sperm. Schenk et al. [4] suggested multiple inseminations totaling $>20 \times 10^6$ sexed frozen-thawed sperm might be advantageous in Holstein heifer donors. However, more studies are warranted to optimize AI protocols in MOET programs to improve embryo production because of extra costs associated with the sexed product and the reported compromised fertility of sexed sperm [1,7].

The aims of our study were to: (1) evaluate the production efficiency of female embryos from superovulated Holstein heifers using sexed frozen-thawed, sexed unfrozen and unsexed frozen-thawed bull sperm, (2) determine the effects of donor parity, number of inseminations and individual bulls on embryo production using sexed sperm under the field settings, and (3) document pregnancy rates and sex bias of the resulted embryos/calves.

2. Materials and methods

2.1. Semen preparation

The present study used proven Holstein bulls owned by Genetics Hokkaido with acceptable pregnancy rates in the field. X-chromosome bearing sperm (X-sperm) from ejaculates were sorted using XY™ sperm sorting protocols [7]. Processed sperm were packaged in 0.5-mL plastic straws (IMV, France) using egg yolk tris extender (6% glycerol [8]) and then frozen. Batches of straws used in the present study were meeting our criteria of (1) ≥35% progressively motile postthaw as determined visually and (2) ≥90% bias for X-sperm [9].

Frozen-thawed X-sperm from a single bull (bull A) were used in Experiment 1 using a same bull as the source of unsexed frozen-thawed sperm (control). In Experiment 2, sexed frozen-thawed sperm and sexed unfrozen sperm from another bull (bull B) were used. Sorted X-sperm that were extended with egg yolk tris extender without glycerol were used as sexed unfrozen sperm. Unfrozen sperm were packaged in straws and then transported and stored at 4 °C before use. When using unfrozen sperm, superovulated donors were synchronized, and then inseminations were performed on the evening and the next morning of a sorting day. Timing of second inseminations of unfrozen sperm was almost 24 h after semen collections. Frozen-thawed sperm from nine bulls including bulls A and B were used in Experiment 3.

2.2. Donor females, superovulation and artificial insemination

For Experiments 1 and 2, Holstein heifers (12–16 mo old) were used as embryo donors and were treated in a same manner at Zen-Noh ET center (Zen-Noh) in 2007 and 2008. Superovulation was induced by intramuscular administration of eight declining doses of FSH (Antrin® R10; Kawasakimitaka KK, Japan, 12-h intervals over 4 d, 28 mg total) beginning 8–12 d after the onset of standing estrus or 6 d after insertion of an intravaginal progesterone releasing device (PRID®; Ceva Sante Animale, France). Each donor was given two IM doses of prostaglandin F2 alpha (Pronalgon F®, Pfizer, USA, 25 and 15 mg of dinoprost, respectively) along with the seventh and eighth FSH treatment, and the PRID was removed following second prostaglandin treatment. Onset of standing estrus was observed approximately 48 h after the first prostaglandin treatment. GnRH analogue (Spornen®, Kawasakimitaka KK, Japan, 100 μg of fertirelin acetate) was administered IM at estrus detection. Each donor was inseminated into the uterine body at 12 and 24 h after the estrus detection.

Experiment 1 included 10 heifers randomly assigned into two groups that were inseminated with sexed ($n = 5$) or unsexed ($n = 5$) frozen-thawed sperm from bull A. In Experiment 2, 19 heifers were inseminated with sexed unfrozen ($n = 10$) or sexed frozen-thawed ($n = 9$) sperm from bull B; 5×10^6 sperm/dose were used in both experiments. We used Holstein heifer flushes ($n = 28$) performed at Zen-Noh in 2007 as a control for Experiment 2. Heifers in control group were inseminated twice with unsexed frozen-thawed sperm ($>10 \times 10^6$ sperm/dose) from a variety of bulls.

For Experiment 3, 99 flushes (60 cows and 39 heifers) were conducted on commercial dairy farms. This 3-year study (2005–2008) encompassed the efforts of nine practitioners. Therefore the superstimulation protocols were not identical due to the variety of practitioners involved. Generally, total FSH doses ranged 20–28 mg in heifers and 28–44 mg in cows. Single inseminations of 5×10^6 sperm/dose (18–24 h after the estrus detection), or two inseminations of 2×10^6 (4×10^6 total sperm), 5×10^6 (10×10^6 total sperm) or 10×10^6 (20×10^6 total sperm) sperm/dose (12 and 24 h after the estrus detection) were used.